



Maternal warming influences reproductive frequency, but not hatchling phenotypes in a multiple-clutched oviparous lizard

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ABSTRACT

The understanding of life-history responses to increased temperature is helpful for evaluating the potential of species for tackling future climate change. Herein, adult southern grass lizards, *Takydromus sexlineatus*, were maintained under two thermal regimes simulating current thermal environment and a 4 °C warming scenario to determine the effects of experimental warming on female reproduction and offspring phenotypes. Experimental warming caused females to oviposit earlier and more frequently; however, it did not affect other reproductive traits, including clutch size, egg mass and clutch mass. Accelerated embryonic development and energy accumulation rate might have occurred in warmed females. Maternal warming appeared to increase early embryonic mortality, but did not shift hatchling size and locomotor performance. Embryos of oviparous lizards might be more vulnerable to climate change at early stages than at later stages. The impacts of climate change in oviparous lizards might be adverse in the longer term because of the shift in pre-ovipositional embryo viability, which possibly led to a decreased number of hatchlings.

1. Introduction

Temperature is an important environmental characteristic given its effects on nearly all biological and ecological processes (Angilleta, 2009). Global temperature has increased in the last century, and is predicted to continue to increase by 1.8–4.0 °C by the end of this century (IPCC, 2007). These temperature changes have already affected the distribution and survival of living organisms (Winkler et al., 2002; Thomas et al., 2004; Araújo et al., 2006), which has been observed in various species, from small insects (such as aphids) to large mammals (such as polar bears) (Bale, 1999; Stirling and Derocher, 2012). A few species can expand the distribution range and population size (Chamaille-Jammes et al., 2006; Ljungström et al., 2015), but others may reduce their distribution ranges or go extinct in response to climate warming (Dunbar, 1998; Stirling and Derocher, 2012). For those small-bodied ectotherms with limited dispersal capabilities, the ability to effectively respond to temperature changes directly affects their adaptability to future climate conditions, and determines their survival (Araújo et al., 2006; Paaïjmans et al., 2013).

Phenotypic plasticity of life history, behavioural and physiological traits can facilitate and increase the resistance of animals to climate change (Urban et al., 2014; Seebacher et al., 2015; Gilbert and Miles,

2016). Consequently, plastic phenotypic responses to short-term temperature changes provide useful information for assessing the potential for adaptation to future climate change in small-bodied ectotherms (Chown et al., 2010; Merilä and Hendry, 2014). Many life-history traits (such as body size, oviposition timing, and reproductive frequency) of ectothermic animals can be modified in response to short-term temperature changes (Chamaille-Jammes et al., 2006; Marquis et al., 2008; Lepetz et al., 2009; LeGalliard et al., 2010). These responses are probably plastic and conducive to increasing their resistance to temperature change (Stearns, 1992; Niewiarowski, 1994). In lizards, behavioural modifications of gravid females can partially buffer environmental temperature variations. However, they may still be sensitive to climatic change because, as ectothermic animals, their body temperatures are primarily environmentally determined (Walther et al., 2002). Studies on life-history responses to temperature variations have been conducted in several viviparous species that reveal considerable variations in offspring morphology and performance (Sorci and Clobert, 1997; Shine and Downes, 1999; Zhang et al., 2010; Tang et al., 2012; Wang et al., 2014). However, the effects of the maternal thermal, especially warming, environment on the life history of oviparous species are seldom investigated (Du et al., 2005; Clarke and Zani, 2012; Lu et al., 2013; Schwanz, 2016).

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This study was aimed to investigate the effects of experimental warming on life history traits of a multiple-clutched, oviparous lizard species (the southern grass lizard, *Takydromus sexlineatus*) and to compare life history responses of this species to a warmed environment with those reported for other species. We maintained adult lizards under two thermal regimes (simulating current thermal environment and a 4 °C warming scenario, respectively). Then, eggs produced by females were incubated at two fluctuating temperatures to evaluate the effects of experimental warming on female reproductive output, embryonic development, and hatchling phenotypes. *T. sexlineatus* is small-sized (up to 60 mm snout-vent length, SVL) lizard, widely distributed in southern China, the Indochinese Peninsula and Indonesia (Zhao and Adler, 1993). It typically inhabits high grasslands and females lay eggs in shallow nests with pronounced temperature variations (Xu et al., 2007). Previous studies on a congeneric species, *T. septentrionalis*, showed that exposure of females to different constant temperatures causes changes in reproductive frequency and fecundity, but not in offspring phenotypes (Du et al., 2005; Luo et al., 2010). However, there is limited research on life-history responses of multiple-clutched species to fluctuating thermal environments. On the basis of previous results of maternal thermal effects on lizard reproductive output, embryonic development, and hatchling phenotypes, we predicted that: (1) while being exposed to experimental warming, female *T. sexlineatus* would oviposit earlier and more frequently, and thus alter their reproductive output; (2) egg-hatching success and hatchling locomotor performance would be reduced if experimental warming had exerted an adverse impact on pre-ovipositional (or post-ovipositional) development of embryos.

2. Materials and methods

2.1. Animal collection and husbandry

In early April 2011, we captured a total of 72 *T. sexlineatus* adults (52 females and 20 males) by hand or noose in Zhaoqing (23°03'N, 112°27'E) and transported them to the laboratory in Hangzhou Normal University. Immediately after arrival, the lizards were randomly assigned to one of three terraria (length × width × height: 120 × 50 × 40 cm, 20–24 females and 6–7 males in each terrarium) filled with moist sand and grass, and acclimated to the laboratory environment for three days. A supplementary heating source (a 70-W sunlamp specially designed for reptiles was suspended 20 cm above the ground on one side of each terrarium, and automatically switched on at 08:00 and off at 17:00) was provided to allow the lizards to bask. Two Thermocron iButton temperature loggers (DS1921, MAXIM Integrated Products/Dallas Semiconductor Ltd., Sunnyvale, USA) were placed on the sand surface in each terrarium (under the sunlamp and at the farthest place away from the sunlamp) to record the temperature every 10 min (Fig. 1A). Food, i.e., mealworms (*Tenebrio molitor*) and house crickets (*Achetus domesticus*), and water enriched with vitamins and minerals, were provided *ad libitum*.

Lizards in the Zhaoqing population become active from late March, and females produce multiple clutches with 1–4 eggs per clutch from late April to July (Xu et al., 2007). The air temperature in Zhaoqing shows a significant seasonal variation, with January being the coldest month and July being the warmest month of the year. The mean air temperature in April is approximately 22.6 ± 0.3 °C (data between 2003 and 2010, Meteorological Bureau of Guangdong Province). Therefore, we designed a thermal regime in an artificial atmospheric phenomena simulator (AAPS) room set at 22 °C, which was considered as the environmental temperatures potentially experienced by *T. sexlineatus* in the early period of active season (hereafter non-warmed treatment, 28 females and 11 males). The other thermal regime was designed in another AAPS room set at 26 °C, to mimic the thermal environment after warming of 4 °C from non-warmed treatment (hereafter warmed treatment, 24 females and 9 males). Two fluorescent lamps in

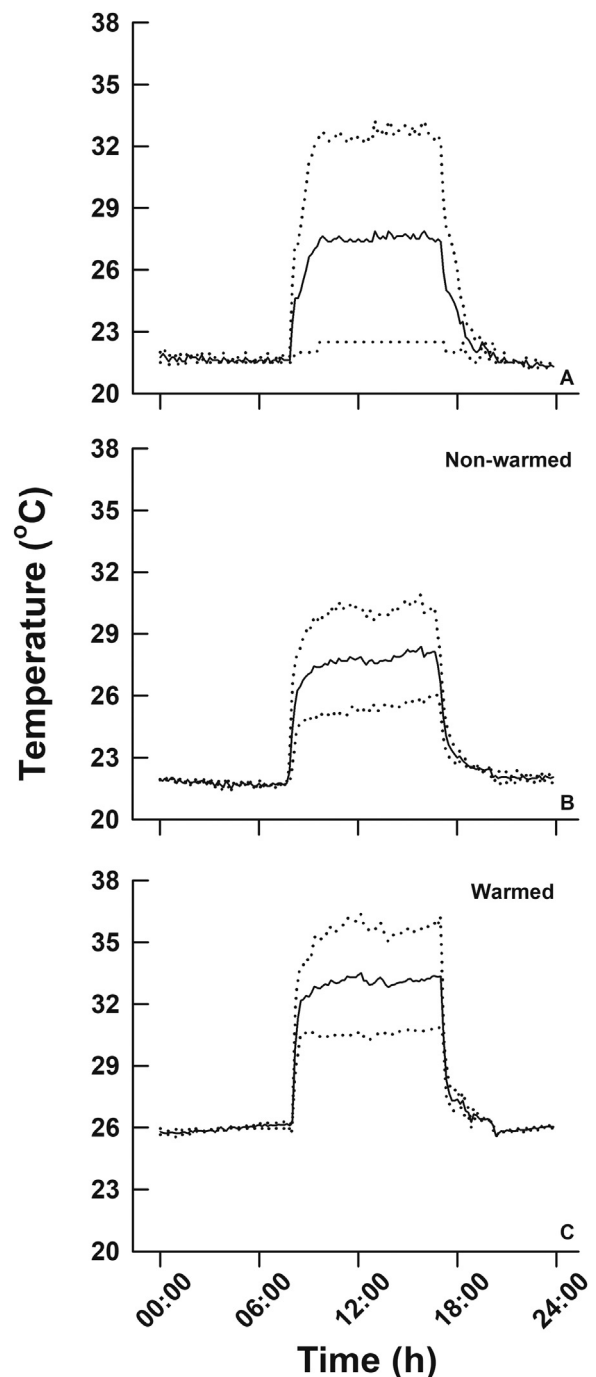


Fig. 1. Mean ambient temperatures in terraria, where adult *Takydromus sexlineatus* were maintained under different thermal environments. The upper and lower dotted lines represented the highest and lowest surface temperatures of substrate in terraria, respectively.

each AAPS room were switched on at 08:00 and off at 17:00. Lizards were maintained in seven terraria (60 × 45 × 40 cm, 7–8 females and 2–3 males in each terrarium), which were then placed in one of two AAPS rooms. A 40-W sunlamp that set to remain on between 08:00 and 17:00 was suspended 15 cm above the ground on one side of each terrarium. There were daily temperature variations in the two treatments at higher temperatures when the sunlamps were switched on. The mean surface temperatures were 24.2 and 28.7 °C (ranges of 21.5–30.8 and 25.5–35.9 °C) in non-warmed and warmed treatments, respectively (Fig. 1B, C). The mean surface temperature did not differ among different terraria in both treatments (non-warmed: $F_{3, 429} =$

1.74, $P = 0.159$; warmed: $F_{2, 286} = 1.37$, $P = 0.256$).

2.2. Female reproduction

We palpated the abdomens of females every 5 days. Females with oviductal eggs were transferred individually into $20 \times 15 \times 20$ cm plastic terraria filled with up to 5 cm of moist sand. A 20-W spotlight was mounted on top of each terraria. The mean surface temperatures of egg-laying terraria were 23.1 (24.6°C at day and 21.8°C at night) and 27.5°C (29.3°C at day and 26.2°C at night) in non-warmed and warmed treatments, respectively. Food and water were also provided for females in egg-laying terraria. The mean time that females remained in egg-laying terraria was $2.4 (\pm 0.1)$ days, with a range of 1–6 days. These egg-laying terraria were inspected at least thrice daily for freshly laid eggs. All eggs were collected and weighed within 2 h post laying. Post-ovipositional females were measured for SVL, weighed, and individually marked using a nontoxic paint before they were returned to the communal terraria. The experiment was terminated at the end of the reproductive season (mid-July). Four females in the warmed treatment died during the experimental period, so the corresponding data were excluded from further analyses. Within each clutch, clutch mass was calculated as the sum of individual egg masses; relative clutch mass (RCM) was calculated by dividing clutch mass by post-ovipositional maternal body mass. Post-ovipositional maternal body condition was calculated as the residual of the regression from log-transformed body mass relative to log-transformed SVL. Clutch frequency was the observed number of clutches, seasonal fecundity and total clutch mass was calculated as the total number and total mass of eggs produced by each female during the entire experimental period.

2.3. Egg incubation

Only eggs from the first clutches were included in this study for comparing egg incubation and hatchling phenotypes, because only few eggs from later clutches were hatched. A total of 53 fertilized eggs were numbered individually and incubated in covered plastic jars (50 mL) filled with moist vermiculite (water potential of -12 kPa, dried vermiculite:water = 1:2). These jars were placed inside two programmable incubators (Ningbo Life Science and Technology Ltd., Ningbo, China) set at 24 ± 4 and $28 \pm 4^\circ\text{C}$ to mimic the thermal environment around potential nests (the mean soil temperature under grass cover in May was about 24°C) and that with the warming of 4°C . Eggs from a single clutch were randomly assigned into the two temperature treatments to minimize clutch effects. The jars were shifted daily among shelves according to a predetermined schedule to minimize the potential effect of thermal gradients inside the incubator.

2.4. Hatchling traits

Upon emergence, each hatchling was collected, weighed and measured for their SVL and tail length. We estimated maximal sprint speed of hatchlings at a body temperature of 28°C , which was achieved by placing them in an incubator at that temperature for 30 min before testing. We chased individual lizards down a 1.5-m racetrack with one transparent side, enabling lateral filming with a Panasonic NV-MX3 digital video camera (Beijing, China). Each lizard was forced to run three times with a minimum of 1-h rest between successive trials. Later, the video clips were transferred to a computer and examined for the sprint speed during the fastest 20-cm interval using the MGI VideoWave III programme (MGI Software Co., Canada). For each individual, the maximal value of three measurements was used for statistical analysis. All hatchlings and their parents were released at the parental capture sites after completing the analysis of hatchling locomotor performance. All experimental procedures complied with the current laws on animal welfare and research in China, and were approved by the Animal Care and Ethics Committee of Hangzhou Normal University.

2.5. Statistical analysis

Female reproductive traits (except for clutch mass) and hatchling traits were independent of maternal size (SVL or body mass, all $P > 0.05$). Student's *t*-test was used to test for differences in reproductive female size, clutch frequency and oviposition time between maternal treatments. A multivariate analysis of variance (MANOVA) was used to test for differences in female reproductive traits (clutch frequency, seasonal fecundity and total clutch mass, or post-oviposition body mass, clutch size, egg mass, clutch mass and RCM, as dependent variables) between maternal (non-warmed vs warmed) treatments, and between the first two clutches. Multivariate analysis of covariance (MANCOVA) with initial egg mass as the covariate was used to test for differences in hatchling traits (body mass, SVL and tail length as dependent variables) between maternal treatments and between incubation treatments. Two-factor analysis of covariance (ANCOVA) with maternal SVL as the covariate was used to test for differences in clutch mass between maternal treatments and between the first two clutches. Two-factor analysis of variance (ANOVA) and ANCOVA with hatchling SVL as the covariate was used to test for differences in incubation length of eggs and sprint speed between maternal treatments and between incubation treatments, respectively. Pearson and Cochran-Mantel-Haenszel (with incubation temperature or maternal treatment as the stratification factor) Chi-squared (χ^2) tests were used to analyze the effect of temperature regime on female reproductive success and egg-hatching success, respectively. We confirmed variable normality using the Kolmogorov-Smirnov test and variance homogeneity using Bartlett's (at univariate level) or Box's M (at multivariate level) test prior to parametric analyses.

3. Results

3.1. Female reproduction

Oviposition occurred between late April and mid-July. Approximately 67% (32/48) of the females produced at least one clutch during the experiment. The proportion of reproductive females was higher in the non-warmed treatment than in the warmed treatment; however, this difference was not statistically significant (71.4% vs 60.0%, $\chi^2 = 0.69$, $df = 1$, $P = 0.408$). The SVL of reproductive females did not differ between treatments ($t = 0.76$, $df = 30$, $P = 0.454$, Fig. 2A). Seasonal reproductive output showed a marginal difference between treatments (Wilks' $\lambda = 0.76$, $F_{3, 28} = 2.97$, $P = 0.049$), with more clutches produced in the warmed treatment ($t = 2.34$, $df = 30$, $P = 0.026$) (Fig. 2B–D). Both the first (4.6 days) and second clutches (3.4 days) of warmed females were produced earlier than those of non-warmed females (time interval between the dates of female capture and their first clutches: $t = 2.31$, $df = 30$, $P = 0.028$; time interval between the dates of the first and second clutches: $t = 2.18$, $df = 21$, $P = 0.041$, Fig. 3).

For the first two clutches, the effects of treatment (Wilks' $\lambda = 0.93$, $F_{5, 47} = 0.68$, $P = 0.638$), clutch (Wilks' $\lambda = 0.85$, $F_{5, 47} = 1.60$, $P = 0.179$) and their interaction (Wilks' $\lambda = 0.96$, $F_{5, 47} = 0.40$, $P = 0.847$) on reproductive traits were not significant (Fig. 4). No significant difference in clutch mass between treatments ($F_{1, 50} = 0.08$, $P = 0.785$) or clutches ($F_{1, 50} = 0.38$, $P = 0.542$) remained when maternal SVL was held constant (Fig. 4E). Post-ovipositional maternal body condition did not differ between treatments ($F_{1, 51} = 0.22$, $P = 0.638$) and between clutches ($F_{1, 51} = 2.57$, $P = 0.115$) (Fig. 4B).

3.2. Egg incubation

Increased maternal temperature during pregnancy caused a decrease in egg-hatching success ($\chi^2 = 5.31$, $df = 1$, $P = 0.021$), despite no difference for eggs incubated at $28 \pm 4^\circ\text{C}$ ($\chi^2 = 0.15$, $df = 1$, $P = 0.695$). Incubation temperature did not affect egg-hatching success

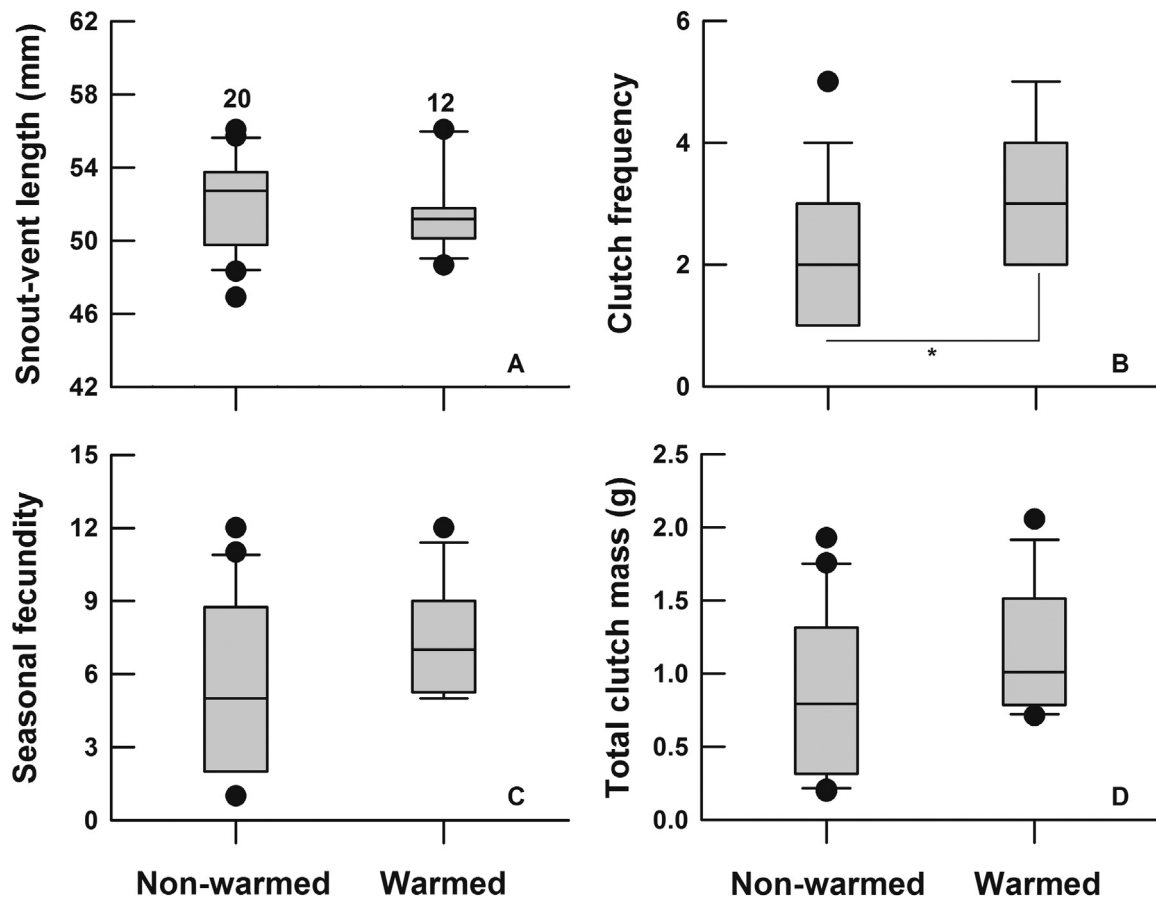


Fig. 2. Seasonal reproductive output of female *Takydromus sexlineatus* maintained under different thermal environments. For boxplots, the horizontal line within the box represents the median, the top and bottom borders of the box represent 25th and 75th percentiles, the whiskers (error bars) represent 10th and 90th percentiles, and filled circles represent outliers.

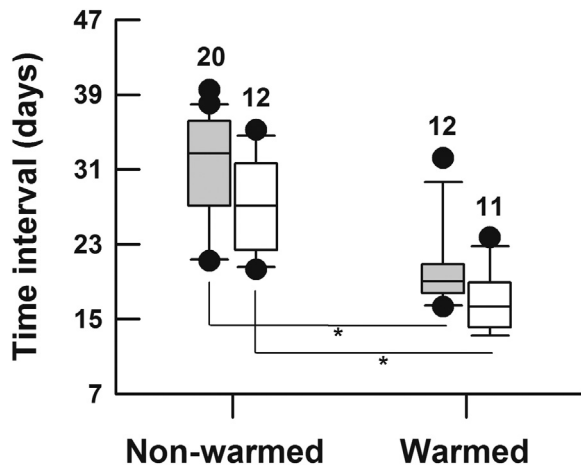


Fig. 3. Time intervals between the dates of female capture and their first clutches (gray-filled boxplots), and between the dates of the first and second clutches (white-filled boxplots) in female *Takydromus sexlineatus* maintained under different thermal environments. Numbers above the error bars are sample sizes. See Fig. 2 caption for explanation of boxplots.

($\chi^2 = 0.09$, $df = 1$, $P = 0.771$) (Table 1). The duration of the incubation period was significantly affected by incubation temperature ($F_{1, 24} = 1737.42$, $P < 0.0001$), but not by maternal thermal environment ($F_{1, 24} = 0.12$, $P = 0.729$) and by its interaction with incubation temperature ($F_{1, 24} = 0.58$, $P = 0.453$) (Table 1).

3.3. Hatchling traits

Hatchling size was not affected by maternal thermal environment (Wilks's $\lambda = 0.89$, $F_{3, 21} = 0.81$, $P = 0.467$), incubation temperature (Wilks's $\lambda = 0.78$, $F_{3, 21} = 1.93$, $P = 0.156$), nor their interaction (Wilks's $\lambda = 0.91$, $F_{3, 21} = 0.72$, $P = 0.552$) (Fig. 5A–C). An ANCOVA revealed no significant differences in maximal sprint speed of hatchlings between maternal treatments ($F_{1, 21} = 0.01$, $P = 0.969$) and incubation temperatures ($F_{1, 21} = 0.06$, $P = 0.808$) (Fig. 5D).

4. Discussion

As reported in other species of lizards (Du et al., 2005; Ji et al., 2006; Luo et al., 2010; Clarke and Zani, 2012; Kubička et al., 2012; Lu et al., 2013; Ma et al., 2014; Wang et al., 2014), the timing of oviposition was significantly affected by the thermal environment experienced by gravid females. Consistent with our prediction, female *T. sexlineatus* maintained in the warmer environment laid eggs earlier and more frequently than the females in the cooler environment. Earlier oviposition and/or more frequent clutch production (and thus shorter clutch interval) may result from accelerated rates of energy allocation for clutch formation and embryonic development under warm environments (Ji et al., 2006; Starostová et al., 2012). It is widely known that embryonic development in ectothermic species is sensitive to maternal gestation temperature, depending largely on environmental temperature (Angilleta, 2009). Environmental temperatures affect energy accumulation and allocation in some species of lizards, such as *Sceloporus olivaceus* and *Paroedura picta* (Dutton et al., 1975; Starostová et al., 2012). In *T. septentrionalis*, females use currently acquired energy

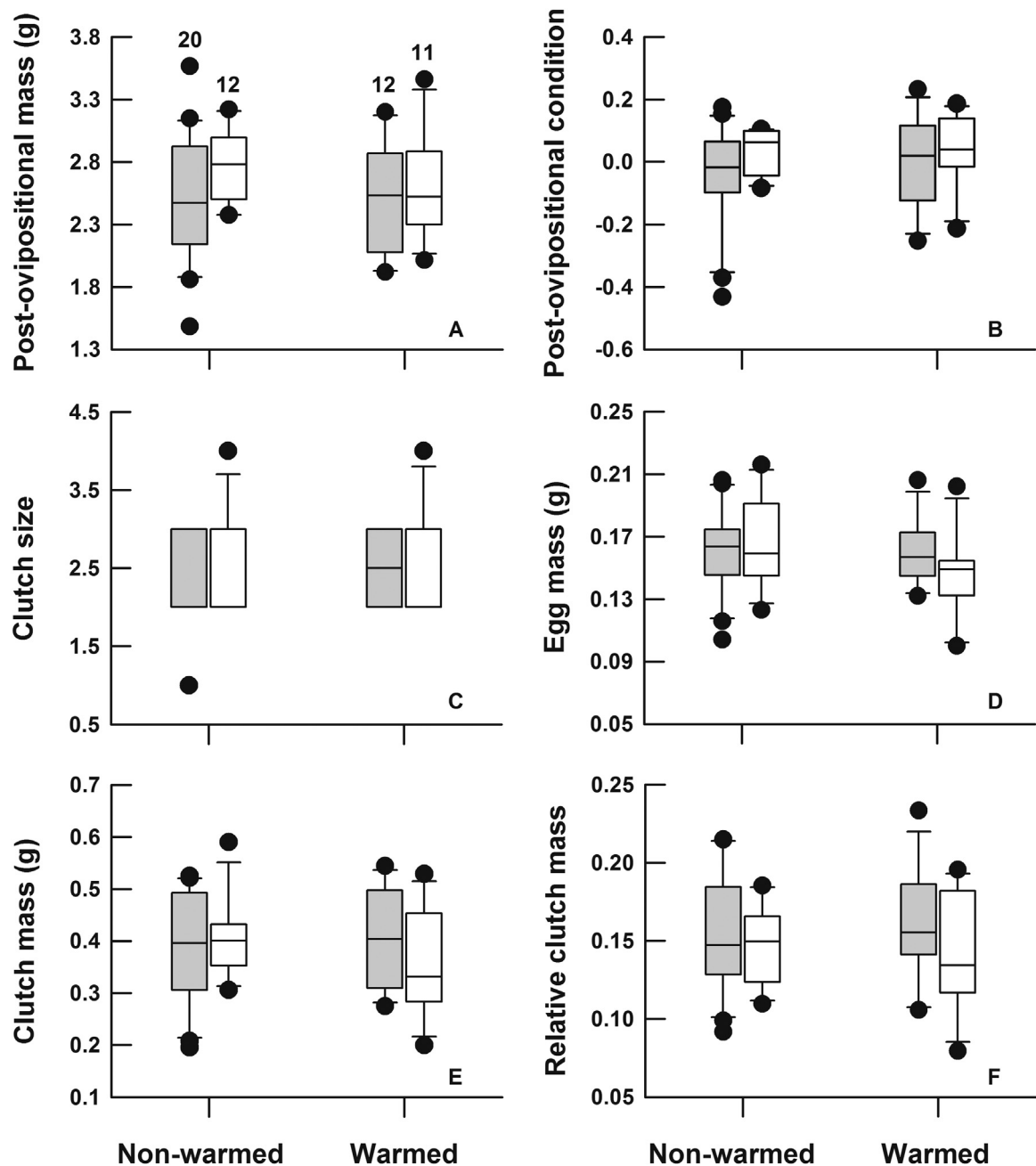


Fig. 4. Reproductive traits of the first (gray-filled boxplots) and second clutches (white-filled boxplots) produced by female *Takydromus sexlineatus* from different thermal environments. See Fig. 2 caption for explanation of boxplots.

Table 1
Incubation length and hatching success of *Takydromus sexlineatus* eggs produced by females from different thermal environments and incubated in different regimes.

Maternal treatment	Incubation temperature (°C)	Incubated eggs	Incubation length (d)	Hatching success (%)
Non-warmed	24 ± 4	15	46.3 ± 0.3 44.5–47.7	80.0 (12/15)
	28 ± 4	13	31.4 ± 0.2 30.6–32.0	53.8 (7/13)
Warmed	24 ± 4	12	46.4 ± 0.7 45.7–47.7	25.0 (3/12)
	28 ± 4	13	31.0 ± 0.2 30.3–31.9	46.2 (6/13)

Data on incubation length were presented as mean ± standard error (range).

from food to produce the first and subsequent clutches of eggs after fulfilling the requirements of maintenance and growth (i.e., income breeding). Despite no change in such energy allocation pattern, warmed females with higher energy intake lay eggs earlier and more frequently than cooled females (Luo et al., 2010). As a congeneric species, income breeding might occur in *T. sexlineatus* as well. This opinion was partially supported by the fact that no significant differences in post-ovipositional body mass and condition were found between clutches. The use of recently acquired energy to fuel reproduction may be widespread in lizards. Even in those species primarily relied on stored energy for reproduction (i.e., capital breeding), recently acquired energy is also used for reproduction from the first to subsequent clutches. For example, a mixed capital-income breeding has been documented in single-clutched lizards (e.g., *Lacerta vivipara*, Bleu et al., 2013), and multiple-clutched lizards as well (e.g., *Amphibolurus muricatus*, Warner

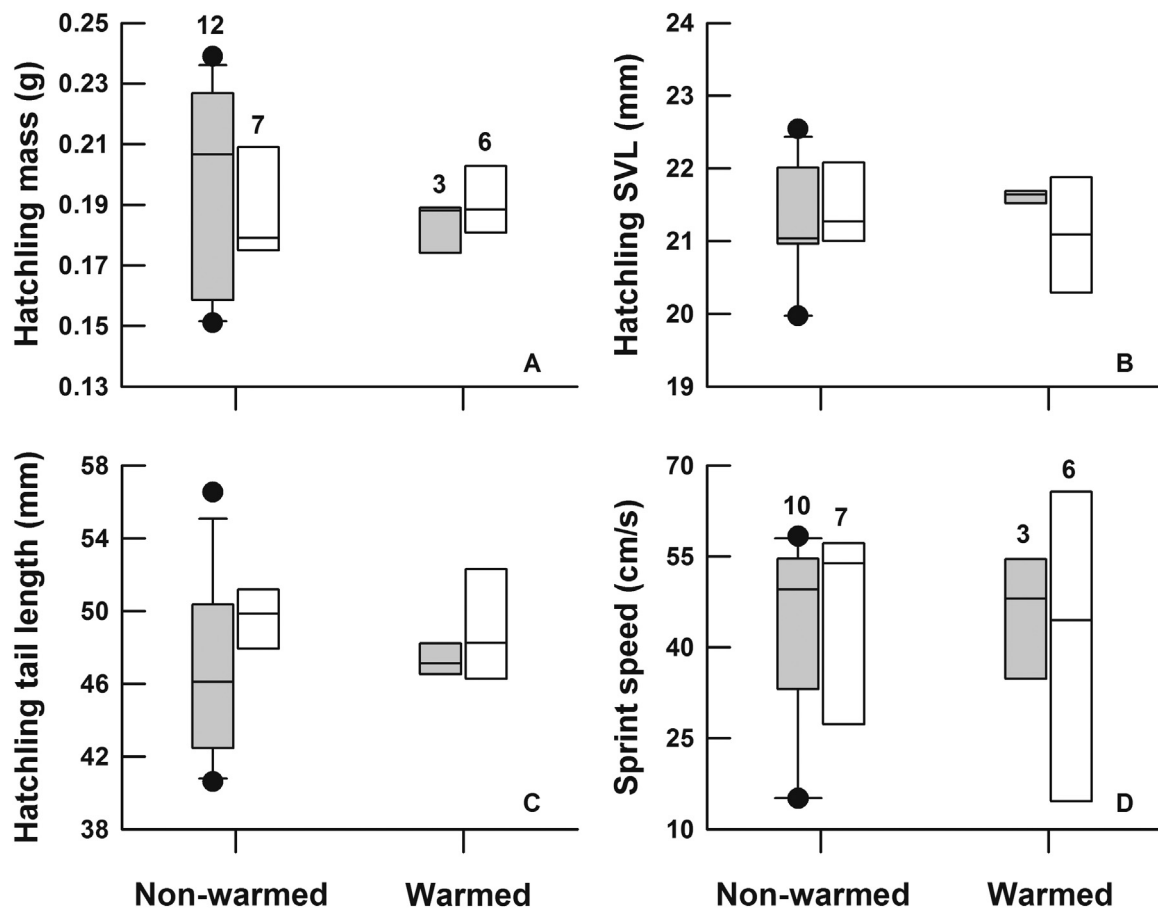


Fig. 5. The size and locomotor performance of hatchling *Takydromus sexlineatus* from eggs produced by females from different thermal environments and incubated in different regimes. Gray-filled boxplots: first clutches; white-filled boxplots: second clutches. See Fig. 2 caption for explanation of boxplots.

et al., 2008).

Generally, the feeding and digestive performance of lizards is thermally dependent (Zhang and Ji, 2004; Lin et al., 2008; Qu et al., 2011). Individuals maintained at warmer temperatures can accrue energy in shorter periods of time by consuming more food and/or improving assimilation efficiency than those at cooler temperatures (Zhang and Ji, 2004; Luo et al., 2010). Although a certain amount of time is required for the formation of a clutch at given temperatures, warmed females should have more sufficient energy to form clutches earlier, allocate energy to eggs more quickly, and thus shorten clutch intervals compared to non-warmed or cooled females. An increased rate of energy allocation to eggs (or egg biomass production), but shortened clutch interval with increasing maternal temperatures has been showed in a multiple-clutched lizard, *P. picta* (Kubička et al., 2012; Starostová et al., 2012).

Despite more clutches being produced by warmed females, differences in seasonal fecundity ($t = 1.91$, $df = 30$, $P = 0.066$) and total clutch mass ($t = 1.54$, $df = 30$, $P = 0.134$) between treatments were not statistically significant. Why did total clutch mass and seasonal fecundity not increase in the warmed treatment as expected? One possible explanation is that there is a trade-off between clutch frequency and within-clutch egg number (especially in later clutches), and laying more clutches in a given reproductive season may result in a decrease in clutch size in later clutches. Unfortunately, this prediction could not be confirmed by the present study due to our limited sample size. Clutch frequency affecting the size and number of egg within clutches has been documented in multiple-clutched lizard species, such as *Eremias argus* (Wang et al., 2011). Contrarily, an increase in seasonal reproductive output owing to increased number of clutches in warm environments is commonly observed in multiple-clutched species (Du

et al., 2005; Luo et al., 2010; Kubička et al., 2012).

Our results showed that the size and number of eggs were insensitive to temperature changes in each reproductive bout of *T. sexlineatus*, similar to other single- or multiple-clutched lizard species (Du et al., 2005; Lu et al., 2013). The egg size of *T. sexlineatus* might be constrained and the maternal energy investment in each egg may be fixed. *T. sexlineatus* females used currently acquired energy to form multiple clutches (including the first clutch) of eggs, regardless of environmental temperatures. They could attempt to produce clutches with a relatively unaltered egg number once sufficient energy had accumulated. However, temperature-dependent egg size is observed in some oviparous lizards, such as *T. septentrionalis* and *Scincella modesta* (Luo et al., 2010; Ma et al., 2014). Such differences among different studies possibly result from diverse embryonic responses to the thermal environment in different lizard populations (Sun et al., 2013). For example, the maternal thermal environment had no effects on egg size and embryonic developmental stages in a southern population of *S. modesta* (Lu et al., 2013). Females may retain eggs in the oviducts beyond the normal time of oviposition under unfavourable environmental conditions, such as dry, low temperature (Braña and Ji, 2007). Cold-treated females laid larger eggs with more advanced embryonic stages than warm-treated females in a northern population of *S. modesta* probably due to extended egg retention (Ma et al., 2014). The maternal thermal environment had no effect on egg incubation length, possibly reflecting similar embryonic developmental stages at oviposition for our different treatments. *T. sexlineatus* females might not retain their eggs for extended periods because the thermal environments of both treatments were not so unfavourable for nesting. Accordingly, the absence of the occurrence of extended egg retention might explain the lack of a between-treatment difference in egg size in this study.

Embryonic development in oviparous species may be affected by the maternally experienced environmental temperature and by nest temperature after oviposition as well (Angilletta, 2009). The maternal thermal environment may have continual effects on embryonic development after oviposition (Lorion et al., 2012; Ma et al., 2014). Consistent with our prediction, the hatching success of eggs produced by warmed females was significantly lower than that of non-warmed females, suggesting that the warming treatment may have altered embryo viability. Why did eggs produced by warmed females suffer from higher mortality than those produced by non-warmed females? The increased mortality of eggs from the warmed regime was unlikely to be resulted from temperature-induced direct injury because an average temperature of 28.7 °C (actually, lower maternal body temperature than this value) should be still within its optimal temperature range for embryonic development (Xu et al., 2014). The possible other explanation is that high-temperature exposure led to increased thermal stress for females, probably due to increased activity level and between-individual interaction under warmed environments. Consequently, the levels of maternal stress hormones (such as corticosterone) were likely elevated, and affected embryo viability and egg hatchability. High-temperature-induced increase in maternal corticosterone levels has been found in some lizard species, such as *Elgaria coerulea* and *E. multicaerulea* (Telemeco and Addis, 2014). Conflicting findings concerning maternal thermal effects on embryo viability have been reported in different oviparous lizards. For example, a maternal warm-treatment reduced egg-hatching success in a southern *S. modesta* population (Lu et al., 2013), yet had no effect in a northern *S. modesta* population (Ma et al., 2014) and *T. septentrionalis* (Du et al., 2005), or a positive effect in *Bassiana duperreyi* (Telemeco et al., 2010). Such inconsistency might result from differences in the optimal temperature range for early embryo development and vulnerability of embryos among populations and species (Braña and Ji, 2007; Andrews and Schwarzkopf, 2012). We speculate that early *T. sexlineatus* embryos in low-latitude regions might be more vulnerable to experimental warming than those of congeneric *T. septentrionalis* in high-latitude regions, similar to patterns observed in the southern and northern populations of *S. modesta* (Lu et al., 2013; Ma et al., 2014).

As found in some oviparous lizard species (Du et al., 2005; Lu et al., 2013), the maternal thermal environment was showed to have no significant effect on hatchling size or locomotor performance. Such result was inconsistent with our prediction. However, thermal conditions before oviposition affect hatchling phenotypic traits (e.g. body size, locomotor performance, and behaviour) in others (Uller, 2008; Telemeco et al., 2010; Clarke and Zani, 2012; Ma et al., 2014; Schwanz, 2016). Accordingly, phenotypic modifications in hatchlings induced by the maternal thermal environment may be not widespread in oviparous lizards. Rather, post-ovipositional thermal conditions can exert a more pronounced effect on hatchling phenotypes than pre-ovipositional thermal conditions (Lu et al., 2013; Clarke and Zani, 2012), although this pattern was not observed in this study. In contrast, significant effects of maternal gestation temperature on the phenotypic traits of offspring are observed in most species of viviparous lizards (Shine and Harlow, 1993; Shine and Downes, 1999; Ji et al., 2006, 2007; Yan et al., 2011; Tang et al., 2012; Wang et al., 2014).

In summary, the maternal thermal environment had a discernible impact on oviposition timing and egg hatching success, but not on other traits for *T. sexlineatus*. Earlier oviposition may have positive ecological consequences; for example, consequent earlier hatchings can increase the probability of hatchling survival in two species of lizards, *L. agilis* and *A. muricatus* (Olsson and Shine, 1997; Warner and Shine, 2007). However, eggs produced by warmed females suffered from higher mortality than ones produced by non-warmed females, indicating a harmful effect of experimental warming of 4 °C on embryonic development. Interestingly, an increase in incubation temperature by 4 °C did not appear to elevate embryonic mortality. These results might reflect a higher embryo's vulnerability to climate warming at pre-

ovipositional stages than at post-ovipositional stages in *T. sexlineatus*. The effects of climate warming on offspring fitness are negative in some reptile species (Lourdais et al., 2004; Dubey and Shine, 2011; Lu et al., 2013; Dayananda and Webb, 2017), but are neutral or even positive in others (Chamaille-Jammes et al., 2006; Ljungström et al., 2015; Schwanz, 2016). In this study, none of examined hatchling traits were affected by the warming of 4 °C before and after oviposition, suggesting a limited impact of climate change on offspring fitness in *T. sexlineatus*. However, the size of this *T. sexlineatus* population might decrease in the future, because the reduced embryo viability (or egg quality) induced by climate warming may cause a perceptible decline in hatchling quantity. On the other hand, individual lizards may adjust their physiological and behavioural performance (e.g., thermal preference, microhabitat utilization, basking frequency and duration) in response to climate warming (Shine and Downes, 1999; Gilbert and Miles, 2016; Telemeco et al., 2017). For example, pregnant lizards under relatively warm environments tend to decrease basking time and be active earlier, and thus achieve similar body temperatures compared with those under cool environments (Lópezalcaide et al., 2014). Whether female *T. sexlineatus* can use such physiological and behavioural adjustments to buffer the harmful effects of climate warming on female reproduction should be determined in future studies.

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